mM deoxyinosine 5'-triphosphate (nucleotide with weak affinity) and 0.05 mM digoxigenin-labeled deoxyuracil 5'-triphosphate (labeled nucleotide) or in a control reaction solution containing 0.5 mM deoxyadenine 5'-triphosphate and 0.05 mM labeled deoxyuracil 5'-triphosphate. Terminal transferase (2.5 units/μL) was added to the solutions and the reaction mixtures were incubated at 37°C for 15 minutes.

(2) The reaction solutions were then transferred on ice, and stopping solution was added thereto (final concentrations: 10 μg/ml glycogen, 0.2 mM ethylenediaminetetraacetic acid). Then, lithium chloride at a final concentration of 0.4 mM and 3 volumes of ethanol were added to the solutions, and the mixtures were incubated at -30°C for 2 hours.

(3) The mixtures were centrifuged at 12,000 g to precipitate DNA. The precipitated DNA was washed with 70% ethanol, and then dried. The dried labeled DNA was dissolved in 100 μL of water.

Example 2 Immobilization of target gene

Immobilization of plasmid DNA on nylon membrane was carried out as follows. A target DNA of about 0.1 ng/μL was heated at 96°C for 10 minutes. This DNA sample was then rapidly cooled down with ice. The DNA was spotted in a quantity of 1 μL/dot onto a nylon membrane for nucleotide blotting (Boehringer). The nylon membrane was wetted with 2x SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7) and then irradiated with ultraviolet light by a UV Crosslinker (Stratagene), according to the attached protocol to fix the target gene on the nylon membrane. Target DNAs used included the above-mentioned DNA with the accession number X75861 and other 8 cDNAs containing nucleotide sequences unrelated to this DNA. These cDNAs all had a poly(A) tail at the 3' ends thereof.

Example 3 Hybridization with labeled oligonucleotide

The hybridization was performed as follows. First, nylon membranes were subjected to pre-hybridization. Each nylon membrane was incubated in a hybridization solution (6x SSC, 1%(w/v) blocking solution (Boehringer), 0.1%(w/v) N-lauroyl sarcosine, 0.02%(w/v)

sodium dodecyl sulfate) with 0.1 mg/ml poly(A) or without poly(A) at 68°C for 3 hours.

Subsequently, the nylon membranes were subjected to hybridization treatment with a labeled oligonucleotide. A labeled oligo DNA (5 pmol/ml) was added to the hybridization solution and incubated at 60°C for 12 hours. The nylon membranes were soaked in a washing solution (6x SSC, 0.1% sodium dodecyl sulfate) at 60°C for 15 minutes. The washing treatment was repeated 4 times.

Hybridization of the labeled oligonucleotide was detected by a luminescent method using a lumino-detection kit (Boehringer) according to the attached instruction manual. Briefly, alkaline phosphatase-conjugated anti-labeled digoxigenin antibody was allowed to react to labeled oligo DNA on a nylon membrane, and then luminescent substrate for alkaline phosphatase was added onto the membrane for the luminescent signal. The lumino-detection was carried out by visualizing the signal on an X-ray film.

The results are shown in Figure 1. The oligonucleotide which is labeled by 3'-tailing with deoxyinosine 5'-triphosphate as a spacer is capable of specifically hybridizing to a plasmid containing an insert of the same nucleotide sequence of DNA but not to cDNAs of unrelated nucleotide sequence of DNA. On the other hand, the oligonucleotide which is labeled by 3'-tailing with deoxyadenine 5'-triphosphate as a spacer is also hybridized to a plasmid having an insert of cDNA containing unrelated nucleotide sequence of DNA in a non-specific manner which is not due to the sequence of the DNA to be labeled. It is impossible to inhibit the nonspecific hybridization even by previously masking the plasmid with poly(A) oligonucleotide.

Example 4 Northern hybridization carried out by using as a probe an oligo DNA labeled by tailing with inosinic acid

RNA was prepared as follows. Cells of animal culture cell line, NT2 (purchased from Stratagene; cell culture was performed according to the attached instruction manual), were suspended in RNA extraction buffer (0.14 M NaCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.6), 0.5% Nonidet P-40, 10 mM vanadyl-ribonucleoside complexes) and allowed to stand

still on ice for 10 minutes. Then, the solution was centrifuged at 10,000 g at 4°C for 15 minutes. The supernatant was removed. Further, an equal volume of proteinase digestion buffer (0.2 M Tris-HCl (pH 7.8), 25 mM EDTA (pH 8.0), 0.3 M NaCl, 2% SDS) was added to give a cell suspension. Proteinase K was added to the suspension at a final concentration of 400 µl/ml and the suspension was incubated at 37°C for 90 minutes. A phenol/chloroform solution was added to the suspension and the aqueous layer was extracted. Further, the phenol/chloroform solution was again added to the aqueous solution. The resulting aqueous layer was extracted and 2.5 volumes of ethanol was added thereto. The solution was centrifuged at 5,000 g at 4°C for 10 minutes. The resulting precipitate was washed with 70% ethanol and then air-dried to obtain a preparation of total RNA. The total RNA was dissolved in H_2O, and incubated at 65°C for 5 minutes. An equal volume of 2x column-loading buffer (1x column-loading buffer: 20 mM Tris-HCl (pH7.6), 0.5 M NaCl, 1 mM EDTA (pH 8.0), 0.1% sodium lauroyl sarcosinate) was added to the solution. The resulting solution was loaded onto a column of oligo-dT cellulose (Collaborative Biomedical Products) pre-swollen with the column-loading buffer, and then the RNA was eluted from the column. The eluted solution was again loaded onto the column. The same treatment was repeated 3 times in total. The column was washed with 5 volumes of 1x column-loading buffer, and 2 volumes of column elution buffer (10 mM Tris-HCl (pH 7.6), 1 mM EDTA (pH 8.0), 0.05% SDS) were added to the column. The eluted solution was recovered, and 0.1 volumes of 3 M sodium acetate and 2.5 volumes of ethanol were added thereto. The resulting solution was centrifuged at 12,000 g at 4°C for 10 minutes. The precipitate formed was washed with 70% ethanol and air-dried to give mRNA. The obtained mRNA was electrophoresed as follows. The mRNA was dissolved in an electrophoresis sample buffer (4 µl of formamide, 2 µl of formaldehyde, 1 µl of 10x MOPS, 1 µl of H_2O (10x MOPS: 14.9 g/L of MOPS, 6.8 g/L of sodium acetate, 3.7 g/L of EDTA, pH7.0)). The sample was heated at 65°C for 10 minutes and then immediately cooled down on ice. The RNA sample was subjected to electrophoresis with agarose (1 g of agarose, 10x MOPS, 73.3 ml of H_2O, 16.7 ml of formaldehyde). The RNA was transferred from the agarose gel to a nylon filter